A SYNTHETIC TRIPEPTIDE WHICH INCREASES SURVIVAL OF NORMAL LIVER CELLS, AND STIMULATES GROWTH IN HEPATOMA CELLS

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SUMMARY: A synthetic peptide with a structure analogous to a growth promoting human serum tripeptide was found to possess activities which, at nanomolar concentrations, increased the survival of normal hepatocytes from regenerating rat liver and which enhanced growth of a line of cultured hepatoma cells. The synthetic tripeptide (glycyl-histidyl-lysine) also stimulated the incorporation of labeled uridine and thymidine into trichloroacetic acid precipitable material.

INTRODUCTION: We have recently identified a small peptide in human serum which enhances macromolecular synthesis in rat liver cells and in rat hepatoma cells in culture. (1) Nanogram amounts of this native factor added to growth-limiting medium caused prolonged survival of hepatocytes and enchanced growth of a cultured line of rat hepatoma cells.

Amino acid analyses suggested that the active factor was a tripeptide formed by glycine, histidine and lysine. We are reporting that synthetic glycyl-histidyl-lysine has similar biological properties to the native factor.

MATERIALS AND METHODS: All necessary reagents for synthesis of the tripeptide were purchased from Sigma Chemical Co., St. Louis. The synthetic procedure was the solid phase method of Stewart and Young (2) as modified for deblocking by Gutte and Merrifield (3). BOC blocking groups were removed with 30% trifluoroacetic acid (TFA) in CH_2Cl_2. The imidazole side chain of histidine was left unprotected.

The completed tripeptide was separated from the resin with HBr in TFA. After removal of HBr and TFA by flash evaporation the peptide was hydrogenated for 48 hr at atmospheric pressure in 90% acetic acid with a 5% catalyst of palladium on barium sulfate. The catalyst was then removed by centrifugation (3000 g x 30 min) and the acetic acid removed by flash evaporation. The dried peptide was dissolved in 2N acetic acid, extracted 3 times with $CHCl_3$. The peptide was purified from the acid phase on plastic microcrystalline cellulose thin layer chromatography (TLC) plates, as previously described for the biological serum tripeptide. (1) The biological assay procedures were also as published.

RESULTS: The synthetic tripeptide glycyl-histidyl-lysine (GHL) was equal to the biological factor in stimulating RNA & DNA synthesis. (Table I) Maximal effects were achieved by both the native and the synthetic factor at concentrations of 20 ng/ml of medium. Higher concentrations exhibited inhibitory tendencies.

The synthetic tripeptide enhanced growth in hepatoma cells, as did the native factor. (Table I) At 20 ng/ml, the synthetic factor appeared to be more effective than the native factor in stimulating cell growth. (Table I) However, estimates of relative activity are qualified by the fact that the purest preparations of native factor were relatively unstable. Degradation of the biological material was signaled by development of a yellow brown color. Such discolored preparations had an inhibitory effect on cell growth. In contrast, the synthetic tripeptide was stable, and maintained its biological activities ever after prolonged storage.

TABLE I

48 HOUR EFFECT OF BIOLOGICAL AND SYNTHETIC TRIPEPTIDES ON SURVIVAL, GROWTH, AND MACROMOLECULAR SYNTHESIS IN NEOPLASTIC AND NORMAL HEPATOCYTES

ADDITIONS	NEOPLASTIC CELLS (HEPATOMA)			NORMAL HEPATOCYTES		
(ng/ml)	Cell number (X 10^6)	RNA (cpm)	DNA (cpm)	Cell number (X 10^6)	RNA (cpm)	DNA (cpm)
0-(control)	1.21±0.08	2100±100	570±40	1.34±0.10	840±50	110±9
Bio-tripeptide						
0.2	1.32±0.06	2400±130	660±30	1.40±0.17	820±40	118±7
2.0	2.15±0.11	4870±270	1060±90	1.66±0.01	1430±140	145±7
20.0	2.38±0.24	5530±230	1240±30	1.92±0.02	1750±260	190±21
200.0	2.05±0.20	5350±160	1030±70	1.73±0.05	1800±80	175±13
Synthetic tripeptide (Gly-His-Lys)						
0.2	1.29±0.02	2570±180	550±70	1.45±0.05	910±30	107±2
2.0	2.40±0.13	7500±160	1250±90	1.60±0.08	1100±110	140±11
20.0	4.67±0.61	10940±380	2140±220	1.88±0.06	1560±80	212±4
200.0	1.91±0.14	8380±390	1490±40	1.45±0.06	1370±80	135±10

Initial cell concentrations were:neoplastic cells,1.00 x 10^6 cells per flask; normal cells, 2.04 x 10^6 cells per flask. Cells were incubated for 48 hours at 37°C in 5 ml media containing 1% calf serum and the added peptide factor. Cultures were pulsed with 1 microcurie of (^{3}H) uridine or (^{3}H) thymidine four hours before the end of the incubation. All samples were in triplicate.

Survival of normal rat liver cells maintained in monolayer culture for 48 hours averaged 61% for controls, and 92% to 94% for cells in medium containing 20 ng/ml of native or synthetic tripeptide.

DISCUSSION: Growth in most cultured mammalian cell lines requires the presence of serum in medium. There is evidence that small molecular factors may contribute to the growth-promoting properties of serum. Dialyzable molecules have been partially isolated from serum after heating or enzymatic proteolysis which sustain growth in serum-free medium of cell lines which normally require serum for growth. (4-7)

Small molecules from other sources have also been shown to be effective substitutes for serum in growth media. Extracts from tissues subjected to enzymatic proteolysis contain growth-stimulatory activities variously attributed to one or more peptides 200 to 600 daltons in size (8-9), a xanthine-like compound 150 daltons in size (10), and a peptone-derived peptide less than 700 daltons in size (11). Very recently, Shodell and Isselbacher (12) suggested that replication of BHK 21 cells requires a small, dialyzable factor, and a macromolecular factor produced by another growing cell line.

The results reported here demonstrate that a synthetic tripeptide, glycyl-histidyl-lysine, possesses the protective and growth-promoting properties of a factor normally present in human serum. These properties of the peptide may reside in an interaction with cellular DNA. Recent NMR studies of DNA-peptide binding have demonstrated the high affinity of DNA for glycyl-histidyl-glycine, (13) lysyl-tryptophyl-lysine, and lysyl-tyrosinyl lysine (14).

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